

# Molluscicidal activity of vulgarone B against ram's horn snail (*Planorbella trivolvis*)

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**Abstract:** The ram's horn snail (*Planorbella trivolvis* (Say)) is an intermediate host for a digenetic trematode (*Bolbophorus confusus* (Krause) Dubois) that has recently been discovered to be a significant problem in commercial channel catfish (*Ictalurus punctatus* Raf) production ponds in the Mississippi Delta region in the USA. In these catfish ponds, the digenetic life cycle of this parasitic trematode involves two intermediate hosts, the ram's horn snail and the channel catfish, and the final host, the American white pelican (*Pelecanus erythrorhynchos* Gmelin). One approach to eradicate this problem is to disrupt the life cycle of the parasitic trematodes by eliminating the snails. During our search for natural-product-based molluscicides to control the snails in the catfish ponds, vulgarone B, isolated from the steam distillate of the aerial parts of the plant *Artemisia douglasiana* Besser (Asteraceae), was found to be active towards the snails with a  $LC_{50}$  of ca 24  $\mu$ M. Channel catfish toxicity studies indicated a  $LC_{50}$  of ca 207  $\mu$ M. Vulgarone B may be an environmentally acceptable alternative for snail control in aquaculture.

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**Keywords:** molluscicides; *Artemisia douglasiana*; vulgarone B; *Planorbella trivolvis*; *Bolbophorus confusus*

## 1 INTRODUCTION

The ram's horn snail, *Planorbella trivolvis* (Say), is an intermediate host for the digenetic trematode, *Bolbophorus confusus* (Krause) Dubois, that has recently been discovered to be a significant problem in commercial channel catfish (*Ictalurus punctatus* Raf) production ponds in the Mississippi Delta region.<sup>1,2</sup> The life cycle of this parasitic trematode involves the snail, channel catfish and the American white pelican (*Pelecanus erythrorhynchos* Gmelin). These trematodes have a digenetic life cycle that involves two intermediate hosts, the snail and channel catfish. Catfish infested by the parasitic metacercariae develop cysts, have impaired growth, and are prone to other diseases that can weaken and kill the catfish. This problem was first noticed in 1999 in the Mississippi Delta region of the USA, but now it has been documented in the states of Arkansas, Louisiana, Alabama and California. The economic loss to the catfish industry in the USA due to the trematode problem is estimated to be in millions of dollars.<sup>2</sup> Catfish is one of the major farm-raised fish in the USA. At the present time there is no cure or a treatment for the infected fish.

One of the possible approaches to eradicate or control this problem is to interrupt the life cycle of the parasitic trematodes by eliminating the snails, which are essential to the life cycle. A

number of chemically diverse natural molluscicides, including triterpenoid saponins, steroid glycoalkaloids, sesquiterpenes, monoterpenes, iridoids, naphthoquinones and flavonoids have been isolated and identified.<sup>3–5</sup> Natural products in general have an advantage over synthetic products, because natural products have a shorter environmental half-life and hence are less likely to accumulate in the environment, the fish pond or in fish flesh.

In our search for natural-product-based molluscicides to control the snails in the catfish ponds, the sesquiterpene vulgarone B (Fig 1), isolated from the plant *Artemisia douglasiana* Besser (Asteraceae), was investigated. Vulgarone B has been reported as a gastropod repellent, and this has led us to test it for the molluscicidal activity.<sup>6</sup> Plants in the family Asteraceae possess compounds with many biological activities, including phytotoxic, antifungal and insecticidal activities.<sup>7–11</sup> Our preliminary bioassay of vulgarone B and the steam-distilled oil of *A. douglasiana* indicated lethal activity against *P. trivolvis*.

## 2 MATERIALS AND METHODS

### 2.1 Plant material

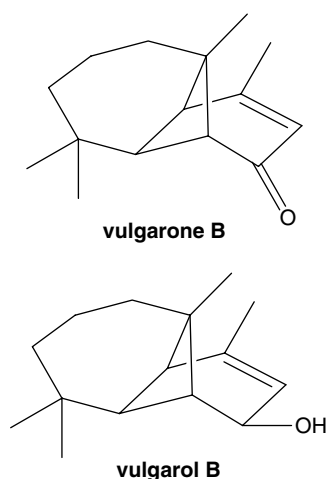
Steam-distilled oil of *A. douglasiana* was provided by Aromagen, Albany, OR, USA. The plants were grown outdoors in Albany, OR, USA.

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**Figure 1.** Structures of compounds tested.

## 2.2 Isolation of vulgarone B

Vulgarone B was isolated from the steam distillate of the aerial parts of *A douglasiana* by silica gel column chromatography followed by crystallization according to Meepagala *et al.*<sup>12</sup>

## 2.3 Synthesis of vulgarol B

Vulgarone B was reduced by lithium aluminum hydride in diethyl ether to obtain vulgarol B according to Meepagala *et al.*<sup>12</sup>

## 2.4 Bioassay for molluscicidal activity

Molluscicidal activity was evaluated according to the method described by Adesina and Adewunmi, with some modifications.<sup>13,14</sup> Test solutions were dissolved in 95% ethanol (500  $\mu$ l) and diluted with distilled water (500 ml). Groups of snails (10 snails per group) were placed into 500-ml beakers containing each test solution. Molluscicidal assays were carried out in triplicate, and these were repeated three times. The negative control was comprised of 95% ethanol (500  $\mu$ l) and distilled water (500 ml). Each test solution was subsequently replaced with distilled water after 24 h and snail mortality for each 24-h period after treatment was recorded for 3 days. Preliminary bioassays of the crude steam distillate were evaluated at 50 and 100 mg liter<sup>-1</sup>. Pure compounds were evaluated in a dose-response format at 0, 10, 30, 50, 100, 150, 200 and 300  $\mu$ M concentrations. Additional molluscicidal assays were carried out as described above in catfish pond water and shallow well water.

## 2.5 Acute toxicity of vulgarone B to channel catfish *Ictalurus punctatus* fingerlings

A short-term (24-h) range-finding test was conducted with vulgarone B. Three channel catfish fry were placed in each of six glass aquaria containing 3 liters of filtered (750  $\mu$ m filter) pond water. Vulgarone B (830 mg) was dissolved in 95% ethanol (1 ml), diluted in distilled water (10 ml), and added to the aquaria at concentrations ranging from 7.8 to 781  $\mu$ M (1.7–170.3 mg liter<sup>-1</sup>). Concentrations less

than 124.8  $\mu$ M (27.2 mg liter<sup>-1</sup>) did not kill fry in 24 h, while concentrations greater than 312.4  $\mu$ M (68.1 mg liter<sup>-1</sup>) caused 100% mortality.

The definitive tests (24- and 48-h, static, single compound, acute toxicity tests) were arranged in a geometrically spaced series of five concentrations using a dilution factor of 0.75 [range: 117.9–372.5  $\mu$ M (25.7–81.2 mg liter<sup>-1</sup>)]. Vulgarone B (2.62 g) was dissolved in 95% ethanol (2 ml) and diluted in distilled water (100 ml). The test consisted of three replications with five channel catfish fingerlings (mean total length 109 mm, SEM = 6.8; mean weight 9.08 g, SEM = 1.68) per replication. Catfish were distributed randomly among the five chemical concentrations and the controls. Exposure chambers were glass aquaria containing 3 liters of filtered (750  $\mu$ m filter) pond water (alkalinity = 231 mg liter<sup>-1</sup> as CaCO<sub>3</sub>, hardness = 312 mg liter<sup>-1</sup> as CaCO<sub>3</sub>, temperature = 21.4 °C, pH = 8.32).

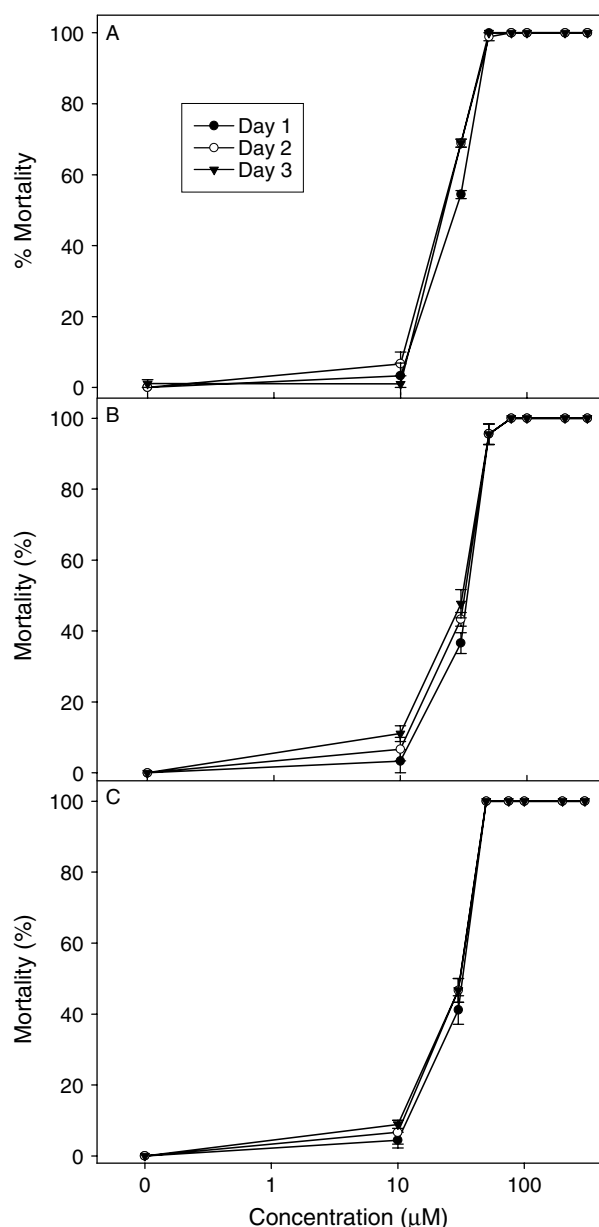
End-points for the tests were death of the organisms. Spearman–Karber analysis and probit analysis with log<sub>10</sub> transformations on dose were used to determine 24- and 48-h LC concentrations using ToxStat (West Inc, Cheyenne, Wyoming, USA).

## 3 RESULTS AND DISCUSSION

Molluscicidal bioassay results demonstrated that vulgarone B had molluscicidal activity with LC<sub>50</sub> of ca 24  $\mu$ M, whether in distilled, pond or well water (Fig 2). The lethal effect of vulgarone on the snails was evident within less than 12 h after treatment (data not shown). Severe hemolysis of snails was observed with vulgarone B during the bioassay. The dose-response curves were the same at 1, 2 and 3 days, further supporting the view that the compound works and dissipates quickly. Dissipation of toxicity apparently has little to do with water quality, since the LC<sub>50</sub> was approximately the same in distilled, pond or well water. Thus, volatilization may be more important than microbial degradation in the dissipation of vulgarone B in water since it has a vapor pressure of 160 mPa (1.19  $\times 10^{-3}$  Torr) at 25.0 °C.

Vulgarol B (Fig 1), the LiAlH<sub>4</sub>-reduced product of vulgarone B with a  $\beta$ -oriented hydroxyl group, had no activity at the concentrations tested (0–300  $\mu$ M), thus indicating the importance of the  $\alpha$ ,  $\beta$ -unsaturated carbonyl functionality for the molluscicidal activity of vulgarone B.

The LC<sub>10</sub> value (162.8  $\mu$ M) for vulgarone B (Table 1) on catfish fry was about fourfold higher than the lowest concentrations required for complete snail mortality (LC<sub>90</sub> ~38  $\mu$ M for snail mortality). As with snail mortality, there was no increased mortality after one day. There were no differences in the 24- and 48-h LC values (Table 1). Although a four-fold safety factor is insufficient for commercial use with catfish fry, we believe that this concern may be mitigated by the prudent application of vulgarone B to ponds. The habitat for the snails is mainly around



**Figure 2.** Molluscicidal activity of vulgarone B against *Planorbella trivolvis* in (A) distilled, (B) pond and (C) well water.  $LC_{50} \sim 24 \mu M$ .  $LC_{90} \sim 38 \mu M$ . Error bars represent the standard errors of the mean ( $n = 3$ ).

the aquatic vegetation of the pond border, whereas the fish are found mostly in relatively deep water. Thus,

application of vulgarone B to the shoreline, rather than direct application into the pond, might provide good control of snails with a sufficient safety margin to avoid toxicity to fish. Also, more mature fish may be more resistant to the compound.

The exact mode of action of most molluscicides is not completely understood, but some of these compounds might bind to the gill membranes of the snails, thus increasing permeability and leading to the loss of necessary physiological nutrients.<sup>4</sup> Hemolysis may be another mechanism of action since hemolysis was observed in the bioassay of vulgarone B. Since there are Michael addition sites in vulgarone B, nucleophilic addition of vital enzymes may also take place and result in molluscicidal activity.

At the present time there is no effective cure available for the trematode-infected fish, nor is there a good product for the control of snails. Treatment of lime along the shorelines of the catfish ponds has been shown to be an effective method of controlling the snails.<sup>2</sup> The drawback to this treatment is that lime changes the alkalinity of the pond water, which may cause undesirable changes in the pond ecology. Application of a mixture of citric acid and copper sulfate has also been shown to kill the snails, but long-term use may cause accumulation of copper in the pond to undesirable levels.<sup>2</sup> We have tested vulgarone B for phytotoxicity in the laboratory, and found it to be non-toxic to plants at the concentrations that were effective as a molluscicide. Thus vulgarone B, a natural product from a plant may be a more desirable treatment than those currently available, due to its high activity at low concentration, selectivity, and relatively short half-life.

The concentration of vulgarone B in the crude steam distilled essential oil fraction is *ca* 11% by weight.<sup>12</sup> The plant *Artemisia douglasiana*, from which vulgarone B was isolated, is a weed which can be grown easily in many parts of the United States and the world. Thus, utilization of vulgarone B or the steam-distilled essential oil of *A. douglasiana* as a molluscicide or a biocide should be economical. Additional research on fish toxicity, bioavailability, mammalian toxicity and half-life in pond water is needed before it can be considered for use in catfish ponds.

**Table 1.** Acute 24- and 48-h toxicity of vulgarone B to channel catfish fry

Analysis method	Time (h)	Toxicity	Vulgarone B concentration	
			$\mu M$ (95% CI)	mg liter <sup>-1</sup> (95% CI)
Spearman-Kärber	24	$LC_{50}$	207.3 (189.4–226.0)	45.2 (41.3–49.4)
	48	$LC_{50}$	207.3 (189.4–226.0)	45.2 (41.3–49.4)
Probit	24	$LC_{10}$	162.8 (142.2–186.2)	35.5 (31.0–40.6)
		$LC_{50}$	207.3 (189.9–226.6)	45.2 (41.4–49.4)
		$LC_{90}$	264.2 (231.2–301.8)	57.6 (50.4–65.8)
	48	$LC_{10}$	162.8 (142.2–186.2)	35.5 (31.0–40.6)
		$LC_{50}$	207.3 (189.9–226.6)	45.2 (41.4–49.4)
		$LC_{90}$	264.2 (231.2–301.8)	57.6 (50.4–65.8)

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